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The primary walls of cotton fibers contain an ensheathing pectin layer

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Summary. Cotton fiber walls (1–2 days post anthesis) are distinctly bilayered compared to those of nonfiber epidermal cells, with a more electron-opaque outer layer and a less electron-opaque, more finely fibrillar inner layer. When probed with antibodies and affinity probes to various saccharides, xyloglucans and cellulose are found exclusively in the inner layer and de-esterified pectins and extensin exclusively in the outer layer. Ovular epidermal cells that do not differentiate into fibers have no pectin sheath, but are labelled throughout with antixyloglucan and cellulase-gold probes. Middle lamellae between adjacent cells were clearly labelled with the antibodies to de-esterified pectins, however. Similarly, cell walls of leaf trichomes have a bilayered wall strongly enriched in pectin, whereas other epidermal cells are not bilayered and are pectin poor. These data indicate that one of the early markers of fiber and trichome cells from other epidermal cells involves the production of a pectin layer. The de-esterified pectins present in the ensheathing layer may allow for expansion and elongation of the fiber cells that does not occur in the other epidermal cells without such a sheath or may even be a consequence of the elongation process.

Keywords: Cotton fiber; Cellulose; Xyloglucan; Pectin; Elongation.

Introduction

With the advent of techniques for the in vitro growth of cotton fibers (Beasley and Ting 1973), cotton fiber development has served as a model to investigate both cellulose biosynthesis and the differentiation and elongation of the fiber cell (Basra and Malik 1984; Ramsey and Berlin 1976a, b; Ryser 1985; Stewart 1975). This system offers several advantages over other systems. First, the differentiation of the fibers in vitro closely parallels the process in vivo but allows more control, such as manipulating temperatures or other conditions (e.g., Haigler et al. 1991). Moreover, within the ovule, a majority of the fibers grow in near synchrony (Schubert et al. 1973), allowing one to harvest a collection of fibers that represent the same stage ontogenetically. Cotton fibers rarely divide (Van't Hof and Saha 1997), so development of the fiber through secondary-wall formation involves only elongation.

Recently, our laboratory and many others have discovered the utility of antibody probes to various saccharide constituents to determine the presence and distribution of specific carbohydrate epitopes (reviewed in Knox 1997). The development and use of these specific saccharide probes has allowed determination of tissue and cell heterogeneity in cell wall composition that would be impossible by standard biochemical techniques. For example, cell walls which face one surface might have a composition different from the wall on the other surface or a specific tissue layer might have a saccharide epitope present only in that cell type and not others (e.g., Lynch and Staehelin 1992, Freshour et al. 1996). Because the antibodies have relatively unhindered access to the saccharides in the thin sections, a quantitative comparison between the relative distribution and abundance of saccharide in different tissues and after different treatments is possible (Vaughn et al. 1996, Sabba et al. 1999). This technique allows for the determination of alterations in cell plates and other structures, that would otherwise be impossible by standard biochemical procedures (Samuels et al. 1995, Vaughn et al. 1996).

Surprisingly, to our knowledge, there has been no systematic investigation of the developing cotton

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fibers with these antisaccharide probes despite the importance of the cotton fiber. Herein, we present results from our investigations of cotton fibers which have been probed with a battery of these antisera and the affinity probe cellulase-gold. Our data indicate that the cotton fiber has a microheterogeneity of saccharide components, especially de-esterified pectins, that may help explain its ability to elongate compared to the adjacent epidermal cells.

Material and methods

Plant material

Cotton flowers at 0 days post anthesis (DPA) were collected from greenhouse-grown *Gossypium hirsutum* L. cv. DPL 5690 plants. Removal of numerous flower parts (e.g., petals, anthers, and style) occurred before the ovaries were surface sterilized. Ovaries were placed in 100% ethanol for less than a minute, flamed, and placed in a sterile petri plate. Ovules were dissected from the ovaries and placed on a liquid medium of Beasley and Ting (1973), except that 120 mM glucose was used as the sole carbon source. Gibberellic acid (0.5 μM) and alpha-naphthalene acetic acid (5 μM) were required for fiber initiation and growth. Ovules were grown at 34 °C for specific lengths of time as described in the manuscript. Other ovules were dissected from the intact plant at the appropriate number of days after anthesis and used directly for analysis.

Microscopy

Ovule segments with fibers were cut into pieces in a drop of 6% (v/v) glutaraldehyde in 0.05 M piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES) buffer (pH 7.4) on dental wax and transferred to a larger volume of the same fixative in scintillation vials (volume, 20 ml) for 2 h. The samples were then washed twice in 0.10 M cacodylate buffer (pH 7.2) and post-fixed in 2% (w/v) osmium tetroxide in cacodylate buffer for 2 h. The samples were washed in several changes of distilled water and en bloc stained with 2% (w/v) uranyl acetate overnight at 4 °C. Samples were washed in distilled water, dehydrated in a graded acetone series, transferred to propylene oxide, and embedded in a 1 : 1 (v/v) mixture of Spurr's low-viscosity resin and epon. Samples were flat embedded and remounted on plastic stubs to obtain longitudinal sections of fibers. Semithin (0.35 μm) sections were obtained with a sapphire knife and ultrathin (ca. 90 nm) sections with a Delaware diamond knife on a Reichert Ultracut ultramicrotome. Ultrathin sections mounted on 300 mesh copper grids or on slot grids coated with formvar were stained with 2% uranyl acetate and lead citrate, 7 min each, before observation with a Zeiss EM 10 CR electron microscope.

An alternative embedding protocol was used for antigens more sensitive to fixation and embedding protocols (esterified pectins recognized by JIM7 and extensin). Fixation was carried out in 3% (v/v) glutaraldehyde in 0.05 M PIPES buffer (pH 7.4) for 1 h at room temperature. Specimens were washed in two exchanges of PIPES buffer at 4 °C and were dehydrated in a step gradient of ethanol at 4 °C with an exchange to 100% ethanol at –20 °C. LR White resin (Polysciences Inc., Warrington, Pa.) was added in 25% increments over 3 days, with two exchanges of 100% resin. The samples were then returned to room temperature and aggitated overnight on a Red Rocker shaker set at 40% line voltage. The samples were transferred to BEEM capsules together with fresh resin and baked at 50 °C for 1–3 h (depending upon the batch of resin utilized).

Samples of young leaf tissue were prepared for microscopy as described by Pettigrew and Vaughn (1998).

Cytochemistry and immunocytochemistry

For immunocytochemical localizations, sections on 300 mesh gold grids were floated on the following solutions: 1% (w/v) bovine serum albumin in phosphate-buffered saline (PBS-BSA) or 1% (w/v) nonfat dry milk (used only for extensin localizations) in PBS (PBS-milk), 30 min; primary antibody diluted in PBS-BSA, 3 h; PBS-BSA, 4 drops over 10 min; goat anti-mouse immunoglobulin G (IgG), goat anti-rat IgG or Protein A conjugated to colloidal gold (all from EY Labs, San Mateo, Calif.) diluted 1 : 20 in PBS-BSA, 30 min; PBS, 4 drops over 10 min. (Dilutions of the primary antibody were determined empirically by examing a concentration range from undiluted to 1 : 320 [Table 1]. Antibody concentrations that gave background labelling of plastic outside the specimen area or starch with less than 0.3 gold particles per μm^2 were utilized.) The grids were then washed extensively with distilled water before post-staining with uranyl acetate (3 min) and lead citrate (30 s). Quantification of the gold particles was determined for 20 randomly selected micrographs (Sabba et al. 1999).

Cellulase-gold cytochemistry was performed as described by Samuels et al. (1996) except that a cellulase purified from Cellulysin (CalBiochem, San Diego, Calif., U.S.A.) was used as the source (purification protocols described in Sabba et al. 1999) and unconjugated gold particles with a diameter of 15 nm were obtained from EY Labs. Controls included prior incubation of the probe in 1 mg of carboxymethylcellulose per ml or inactivation of the probe by heat treatment to 80 °C.

Antibody and antiserum specificities have been determined by a number of groups and are summarized here and in a previous publication from our laboratory (Sabba et al. 1999). The monoclonal antibodies JIM5 and JIM7 are rat monoclonal antibodies that recognize mostly de-esterified (0–30% esterification) or highly esterified (50–100%) pectin epitopes. The polyclonal antiserum to pectin recognizes primarily the de-esterified polygalacturonic acid (PGA) backbone; localizations in our hands reveal similar localizations with this polyclonal serum and the JIM5 monoclonal antibody. The polyclonal antixyloglucan serum recognizes neither pectin molecules nor cellulose (Moore and Staehelin 1988), whereas the mouse monoclonal antibody recognizes a fucosyl-containing epitope found on most xyloglucans (Freshour et al. 1996). Localizations with these two xyloglucan probes label the same structures (Vaughn et al. 1996), and despite the presence of fucose in some pectin preparations, the monoclonal antibody does not label areas of exclusive pectin accumulation, such as the middle lamellae (Freshour et al. 1996). The callose monoclonal antibody has been utilized in a number of laboratories and appears to be highly specific for structures with high callose concentrations such as cell plates and plasmodesmata (e.g., Vaughn et al. 1996). The extensin rat monoclonal LM1 is the most specific monoclonal for extensin (Smallwood et al. 1994).

Results

Cotton fiber cell wall contains two distinct layers

Cotton fiber cells rapidly differentiate themselves from the surrounding epidermal cells and show evidence of extensive cell elongation even in just 1 DPA (Fig. 1 A). In both cross and longitudinal section

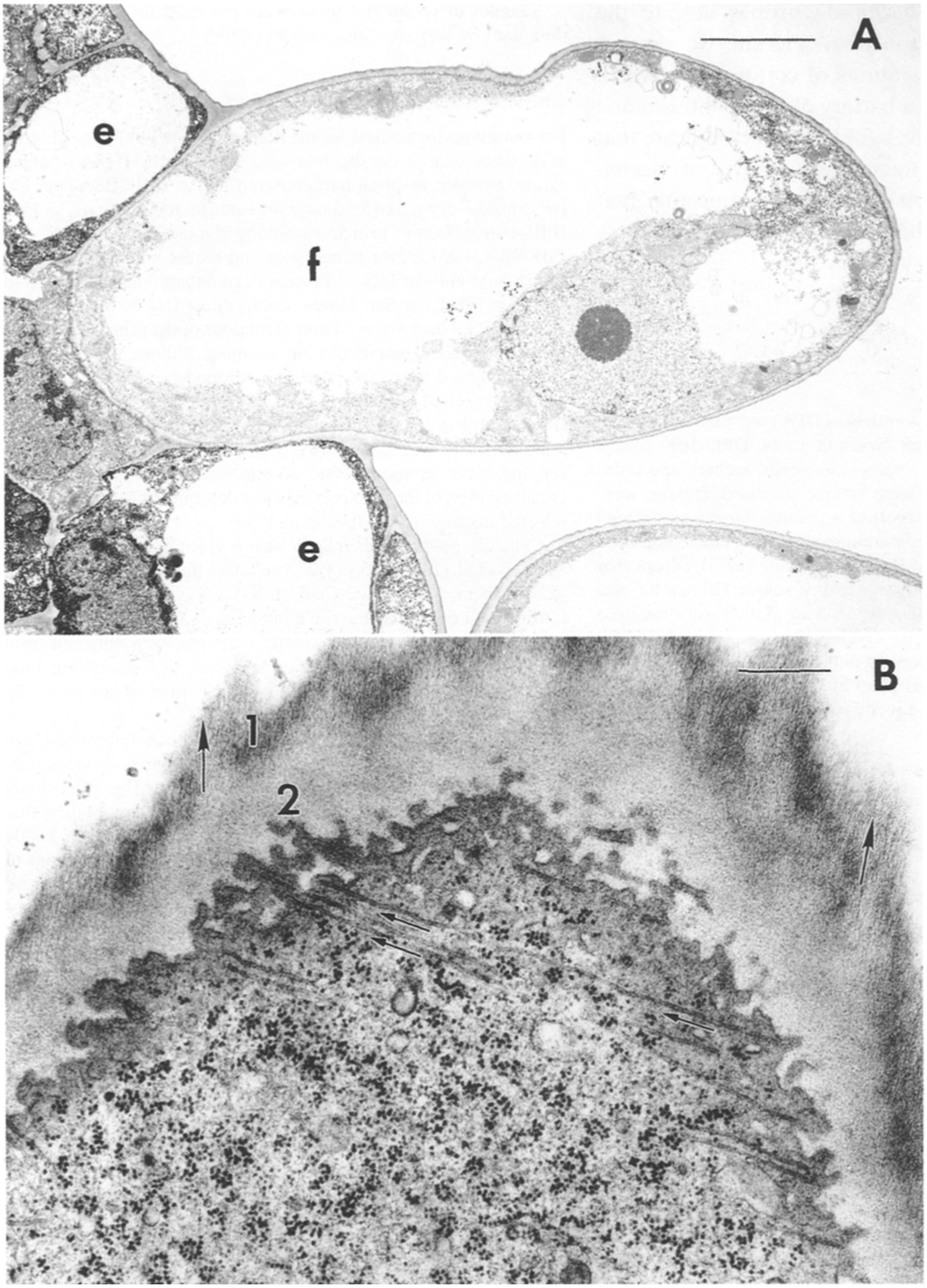

Fig. 1 A, B. Electron micrographs of cotton fiber cells. **A** Low-magnification electron micrograph, 1 DPA cotton ovule culture already displays differentiation of the fiber (*f*) cells from the adjacent epidermal (*e*) cells. **B** In a 2 DPA fiber, two distinct layers of cell wall may be observed, an outer layer (*1*) which is more electron opaque than the inner layer (*2*). The two layers display different orientation of their fibrils, as the inner layer parallels the deposition of the microtubules (arrows with small heads), whereas the outer layer is oriented completely different, nearly perpendicular to the fibrils in the inner layer (arrows with large heads). Bar: A, 5.0 µm; B, 0.5 µm

through the primary wall of the young (1–2 DPA) cotton fiber, two distinct layers are found in the primary cotton fiber wall (Fig. 1 B). The outer layer is more electron opaque and accounts for about a third of the thickness of the wall. This zone is generally amorphous although some orientations of the fiber cells do reveal fibrillar structures, generally more coarse than those that occur in the inner layer. The inner layer accounts for about two thirds of the wall diameter and is finely fibrillar, with the innermost fibrils paralleling the microtubule array (discussed in Seagull 1993), whereas the outer layer is oriented in a direction roughly perpendicular to the axis of the microtubule arrays. These two layers of the wall may be observed throughout the length of the fiber, even to the tip. Similar bilayered cell walls are noted in other published micrographs of cotton, but have not been characterized further (e.g., Ryser 1985, Seagull 1993). A very thin, waxy cuticular layer is also noted, that is continuous across both the fiber and the other epidermal cells and is occasionally observed as a thin line of material which is separated from the outer layer of the fiber. Examination of cotton fibers grown on bolls up to 18 DPA (just prior to substantial secondary-wall production) also revealed a bilayered wall construction, although the outer layer was proportionately less of the volume of the fiber than in the earlier stages described above (not shown). Thus, the bilayered condition is not a consequence of culturing nor is it confined to very early stages of fiber formation.

The two layers of the cotton fiber wall are compositionally unique

The two ultrastructurally distinct layers of wall are also compositionally unique as shown by immunogold and affinity gold analysis. When probed with either polyclonal or monoclonal antibodies to xyloglucan (Fig. 2 A) only the inner wall is labelled, leaving the

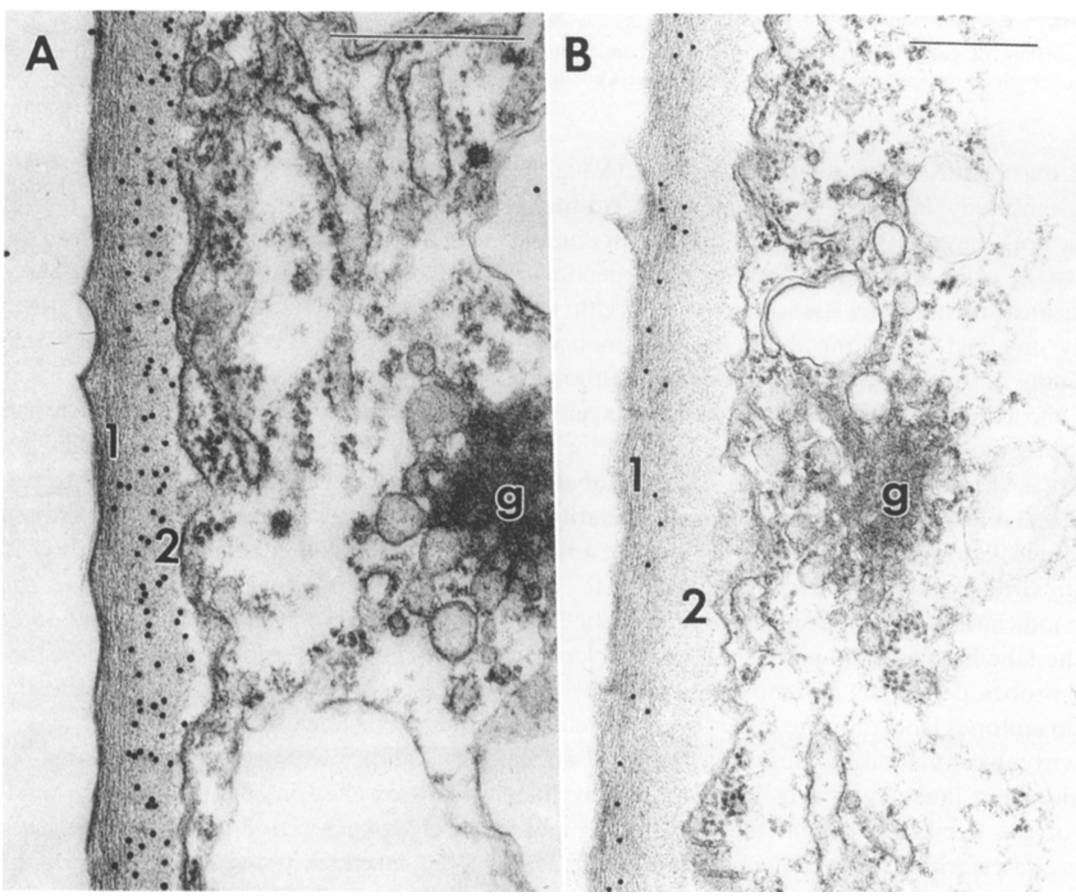

Fig. 2. Immunocytochemical localizations of xyloglucan with polyclonal antixyloglucan (**A**) and polyclonal anti-PGA backbone (**B**) in 2 DPA cotton fibers. When probed with a polyclonal antixyloglucan, only the inner layer (*2*) is labelled, whereas when an adjacent section is probed with the anti-PGA, only the outer (*1*) layer is labelled. *g* Golgi. Bars: 0.5 µm

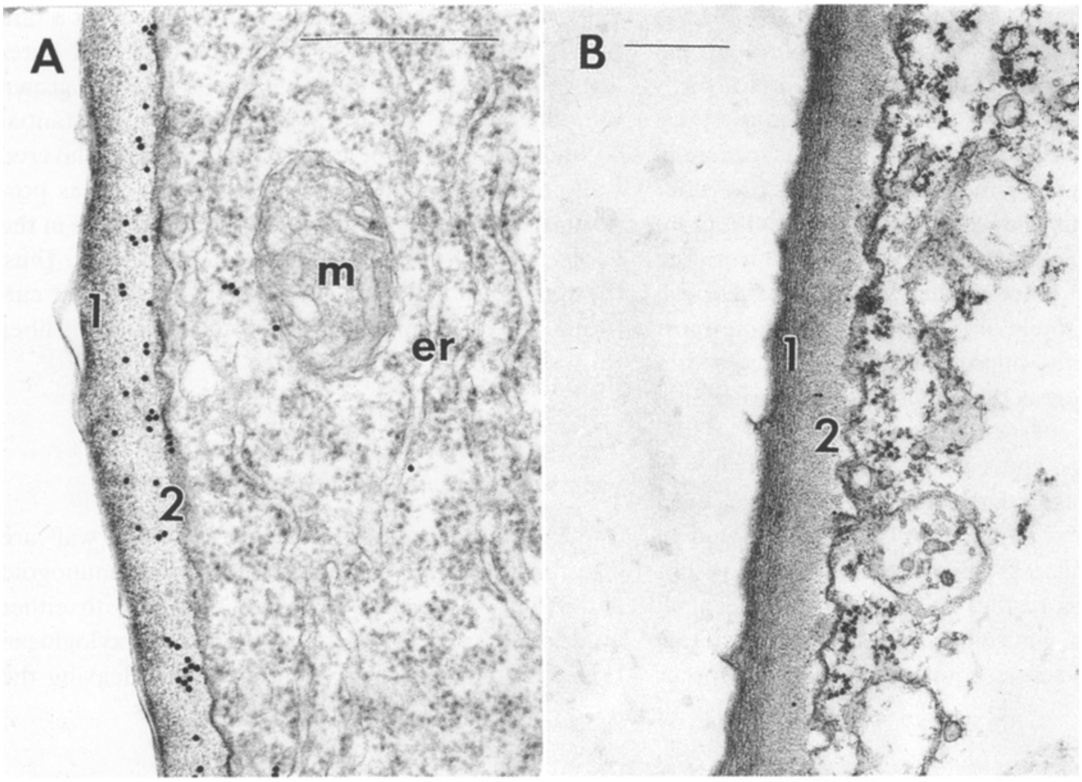

Fig. 3. Affinity-gold labelling of cellulose (**A**) and immunocytochemical localization of callose (**B**) in 2 DPA cotton fibers. Both cellulose and callose are confined to the inner (*2*) layer of wall. *m* Mitochondrion; *er* endoplasmic reticulum. Bars: 0.5 µm

outer, more electron-opaque, layer relatively label-free. Occasionally, antibody labelling occurs in the Golgi vesicles, indicating they are the site of movement of this saccharide to the cell walls (Moore and Staehelin 1986). Xyloglucans are normally associated with cellulose, probably involved in coating the cellulose microfibrils and aiding in their three-dimensional orientation (Carpita and Gibeaut 1993). Similarly, cellulose, as detected with the cellulase-gold probe, only labels the inner areas of the wall, leaving the outer layer unlabelled (Fig. 3 A). Preincubation with the substrate, carboxymethylcellulose, prior to enzymo-gold labelling resulted in virtually no gold particle labelling (not shown), as an indicator of probe specificity.

In contrast to the labelling with the antixyloglucan and cellulase-gold probes, polyclonal and monoclonal antibodies to pectin epitopes label only the outer layer of the wall, with virtually no labelling present in the more electron-translucent inner layer (Fig. 2B). It is likely that most of the pectin present in the outer wall is primarily the de-esterified form, as the reaction with the polyclonal pectin serum is primarily to polygalacturonic acid backbone and the monoclonal JIM5 recognizes predominantly the de-esterified forms (Vaughn et al. 1996, Knox 1997, Sabba et al. 1999). Additionally, treatment of sections on grid with sodium carbonate (which would de-esterify the pectins) resulted in no substantial increase in labelling with either the polyclonal antipectin nor the JIM5 monoclonal in the outer layer of the fiber wall, although some labelling is associated with the inner layer after this treatment whereas it was not found prior to the chemical de-esterification (not shown). Labelling with the monoclonal antibody JIM7 to primarily esterified pectin was just above background levels of labelling in epoxy-embedded samples but in LR White resin-embedded samples most of the labelling was present in both the inner layer and outer layers (Fig. 5 A). These may represent newly synthesized pectins before de-esterification in transit to the electron-opaque pectin sheath.

Labelling of callose with either monoclonal or polyclonal probes resulted in relatively sparse labelling and all label was associated with the inner layer (Fig. 3B). At the interface between the base of the fiber cells and the subtending epidermal cells, plasmodesmata are abundant (Fig. 4A) and the cell wall around the plasmodesmata reacted strongly with both

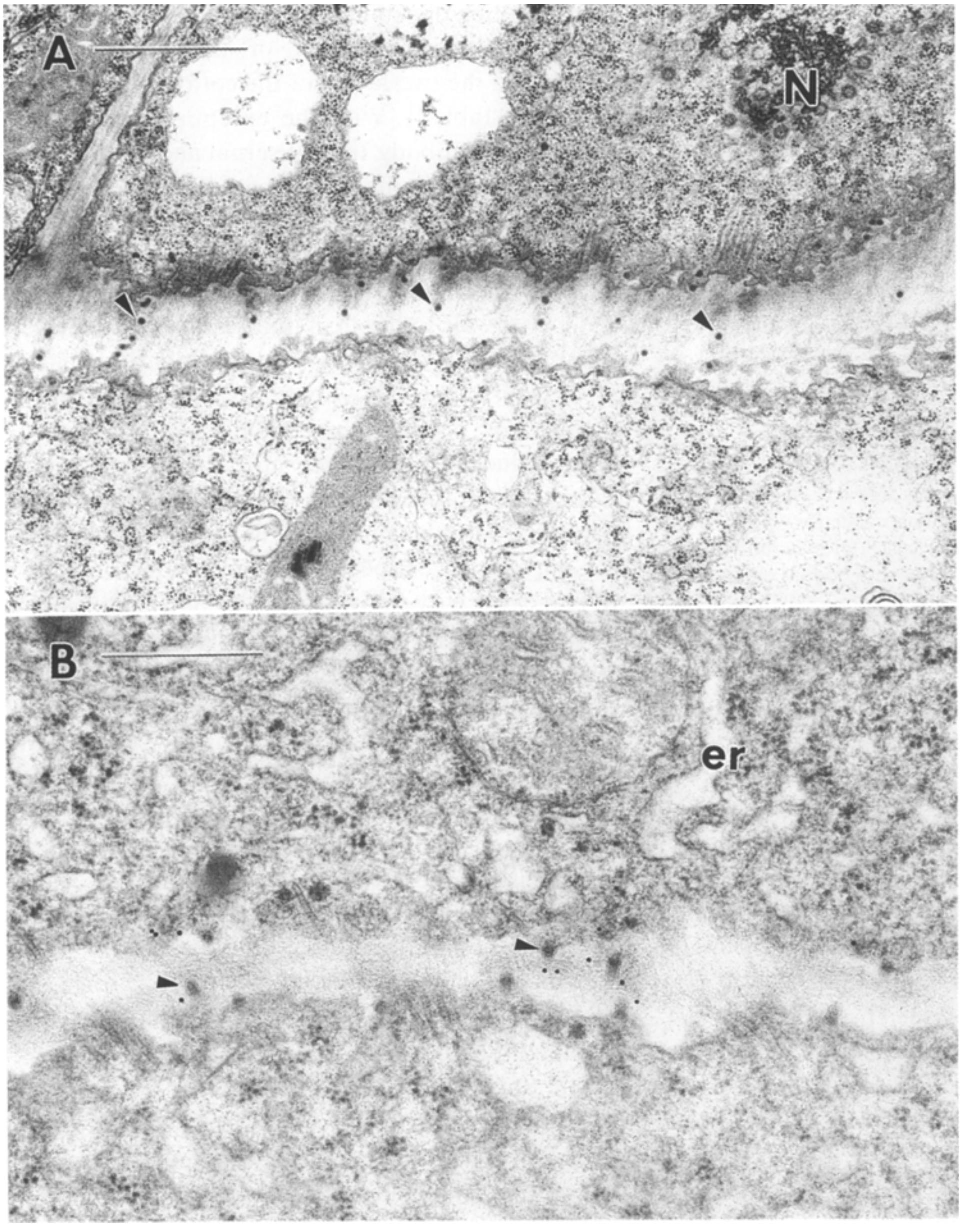

Fig. 4. Electron micrograph (**A**) and immunocytochemical localization of callose (**B**) in the base of the fiber cells of 2 DPA cotton fibers. **A** Cell walls at the base of the fiber are extensively connected to subtending epidermal cells by abundant plasmodesmata (arrowheads). *N* Nucleus. **B** A section similar to that in **A** probed with monoclonal anticallose. Areas of cell wall around plasmodesmata (arrowheads) are distinctly labelled with anticallose. Bar: A, 1.0 μm; B, 0.5 μm

the polyclonal serum and monoclonal antibody to callose (Fig. 4B). Callose has been reported to exist in fibrillar form in older fibers, but only as diffuse layers in earlier stages (Waterkyn 1981), which is in accordance with the sparse and nonpatterned distribution observed in the immunogold localizations for young fibers shown herein.

We have previously reported associations of de-esterified pectins with the wall protein extensin in BY-2 tobacco cells habituated to the cellulose biosynthesis inhibitor herbicide dichlobenil (Sabba et al. 1999), so we wondered if there were similar sorts of pectin-extensin association in developing fiber cells as well. Although probing sections of cotton fibers embedded in epoxy resin resulted in no substantial labelling with any extensin antibody or serum, label was found associated with the outer pectinaceous layer of the fiber for all three extensin antibodies when the tissue was embedded in LR White resin. LM1, which reacts most specifically with extensin of all of the antibodies char-

Table 1. Distribution of immunogold labelling in layers of the cotton fiber

Antibody or probe[a]	% of total labelling[b]	
	Layer 1	Layer 2
Xyloglucan polyclonal (1:20)	8	92
Xyloglucan monoclonal MM-1 (1:40)	4	96
PGA polyclonal (1:20)	96	4
Cellulase-gold (1:20)	14	86
JIM5 monoclonal (1:20)	94	6
JIM7 monoclonal (1:10)	37	63
Callose monoclonal (1:80)	3	97
LM1 (extensin) (1:80)	82	18

[a] Sources and other descriptions of these antisera or monoclonals are given in Sabba et al. (1999) and references therein. In parentheses, the dilution factor of the primary antibody or probe
[b] Gold particle distribution in the outer electron-opaque layer (layer 1) or electron-translucent inner layer (layer 2) of the cotton fiber from 20 micrographs was counted and the totals expressed as a percentage of the labelling in the two layers

acterized to date (Smallwood et al. 1995), clearly labelled the pectin layer of the fiber with little label in the interior portions (Fig. 5 B). The density of labelling with any of these sera was much less than in the dichlobenil-habituated BY-2 cells (Sabba et al. 1999), however. Some extensin label was also detected on the interface between microtubules and the plasma membrane but not elsewhere through the inner layer.

Cell walls in the nonfiber ovule epidermal cells lack both the bilayered wall structure and the presence of an extra band of pectin wall (Fig. 6 A, B). In fact, the distinction between the presence of de-esterified pectin in the wall material in the fiber and the absence in the adjoining epidermal cell was nearly absolute. In the nonfiber epidermal cells, the xyloglucan extends all the way to the cuticle and there is no difference in electron opacity throughout the wall (Fig. 6 A, B). De-esterified pectin was present in the middle lamellae and around the edge of intercellular spaces (not shown), as previously demonstrated in a number of systems (Lynch and Staehelin 1992, Vaughn et al. 1996). Xyloglucans and cellulose occupy much of the wall proper in nonfiber cells (not shown), with callose deposits prominent in new cell plates and around areas of plasmodesmata (Fig. 4 B). Thus, different from the fiber itself, the other walls present in the cotton ovule are biochemically and organizationally similar to the majority of dicotyledonous plant cells (e.g., Lynch and Staehelin 1992, Vaughn et al. 1996).

By counting the immunogold labelling in each layer, a quantitative comparison of the percentage of labelling of the two layers of the cotton fiber wall can be made (Table 1). With the exception of the JIM7 monoclonal antibody, the antiserum and antibodies to pectin (or predominantly de-esterified pectin) label predominantly (>90%) in the outer layer of wall. The JIM7 antibody recognizes primarily methyl-esterified pectin epitopes and label is distributed in both layers of the fiber wall. Other than the labelling of the plasma membrane–wall interface with extensin, the majority of the label in the wall is associated with the ensheathing layer of pectin. The distribution of labelling of antixyloglucans, anticallose, and cellulase-gold probes reveals the preponderance (85–96%) of the label is associated with the inner layer of the cotton fiber wall. We might even suspect an even greater degree of mutual exclusivity since classifying some gold labeling at the edge of the inner–outer border as being in one layer or another is difficult, perhaps accounting for the small amount of label of xyloglucan in the outer layer and pectin in the inner layer. Thus, the quantitative analysis of immunogold labelling firmly establishes the difference in composition between the two layers of the wall as was indicated by the visual observation of the labelling patterns.

Trichomes of developing cotton leaves contain similar pectin sheaths

Because fibers are ontogenetically related to other epidermal appendages such as trichomes, we examined developing trichomes of cotton leaves to determine if they also had an increased pectin content relative to nonexpanding epidermal cells. As for the fibers, the trichome cell walls had a very strong reaction with the de-esterified pectin probes (Fig. 7 A, C), whereas the epidermal cells displayed weak or no reactions (Fig. 7 B). The distinct layering of pectin was less obvious in some trichomes than in others, with internal walls of multicellular trichomes apparently labelled throughout their thickness (Fig. 7 A) or in some cases with the same gradation of reaction across the wall as noted in the fiber cells (Fig. 7 C). Outer walls of multicellular trichomes were most distinctly bilayered as is noted in the fibers. Thus, the asymmetric distribution and increased presence of pectins appears to be a characteristic not just of elongating fiber cells but all epidermal cells in which elongation or differentiation has occurred.

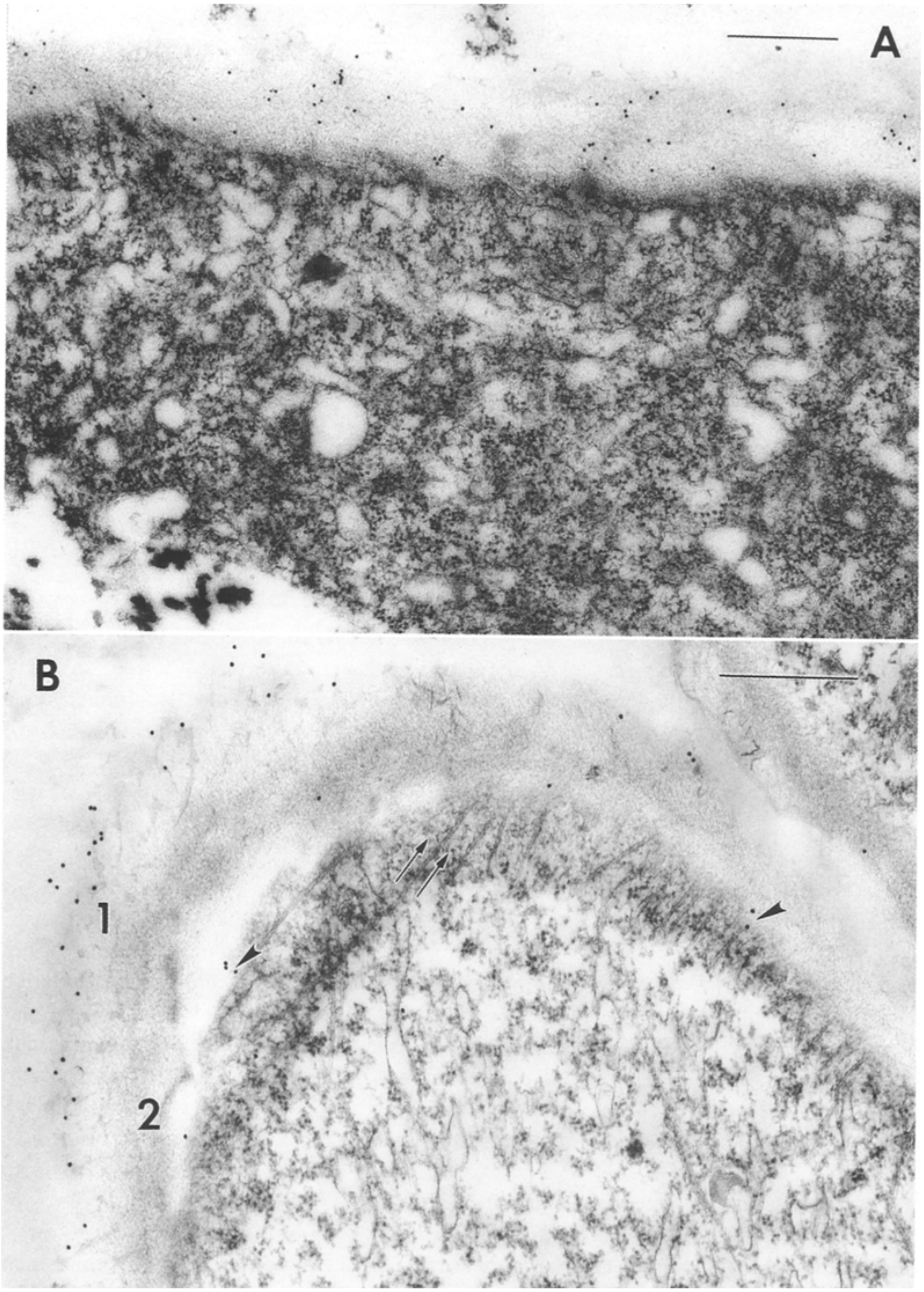

Fig. 5. Immunogold localizations of esterified pectin with the JIM7 monoclonal antibody (**A**) and of extensin with the LM1 monoclonal antibody (**B**) in 2 DPA cotton fibers. **A** Although de-esterified pectin sera and monoclonal antibodies label primarily the outer layer, the JIM7 antibody labels apparently evenly across the entire wall face. **B** The LM1 monoclonal antibody labels primarily layer 1 of the fiber cell but some label is found in layer 2 as well, primarily associated with sites along the plasma membrane where microtubules are associated (arrowheads). Bars: 0.5 μm

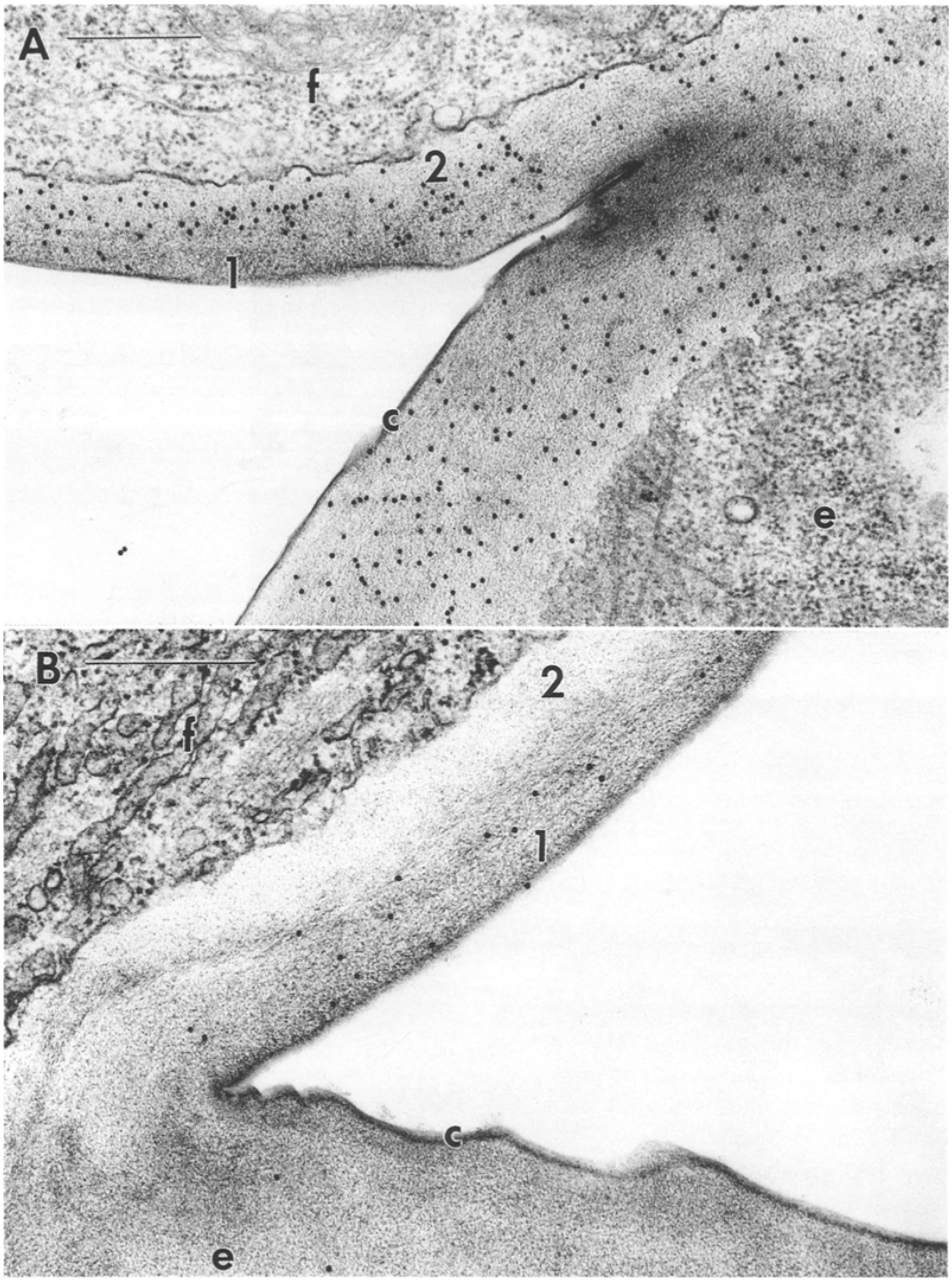

Fig. 6. Side-by-side immunolabelling comparisons between fiber (*f*) and epidermal (*e*) cells in 2 DPA cotton fibers. **A** When cells are labelled with antixyloglucan, the fiber cells are labelled predominantly in the inner (*2*) layer, whereas epidermal cells are labelled throughout, with the exception of the cuticle (*c*). **B** When a similar section is labelled with anti-PGA, the fiber cell is labelled in the outer (*1*) layer of the wall but only very sparsely in the adjacent epidermal cell (*e*). Bars: 0.5 μm

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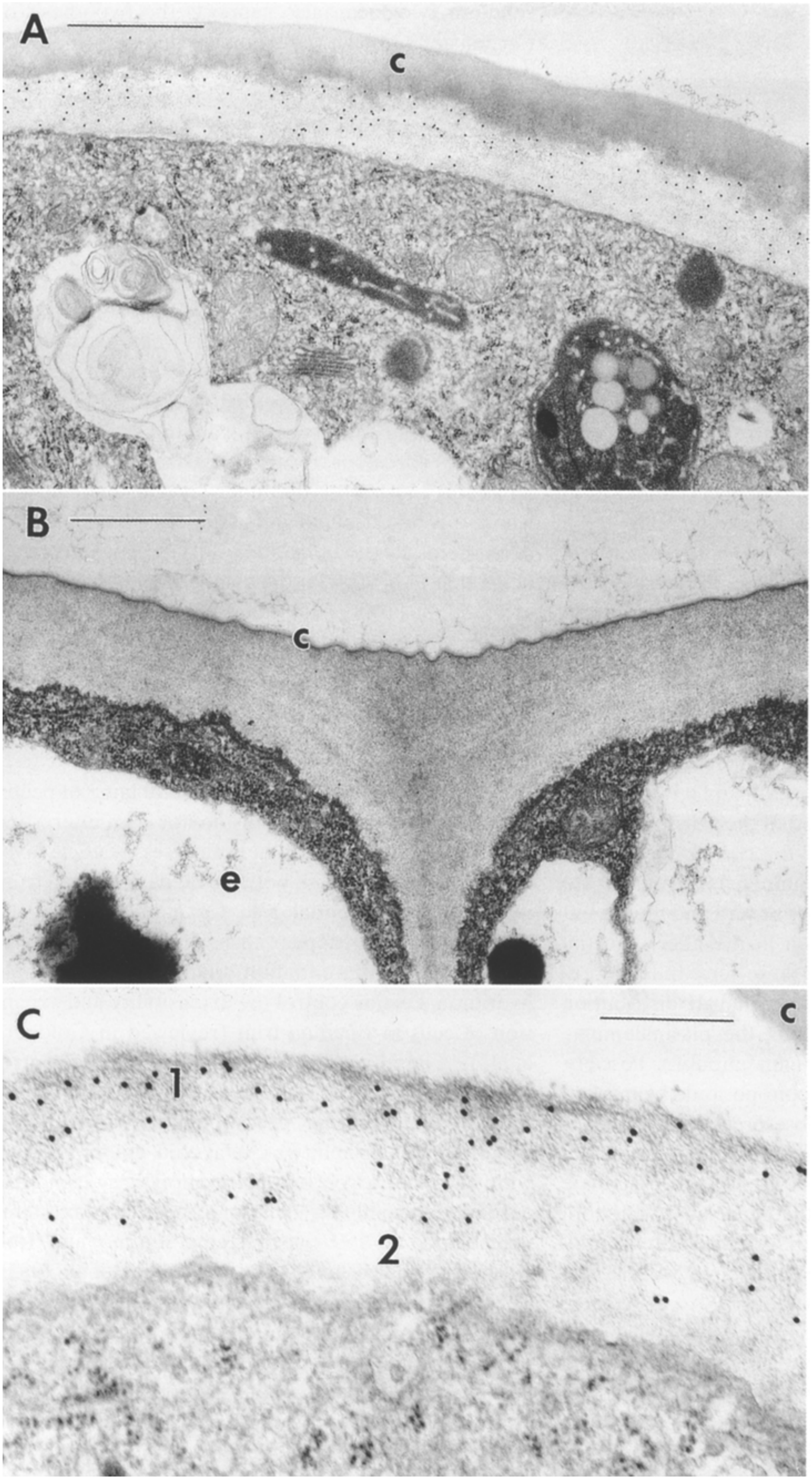
Fig. 7. Localizations of pectin utilizing the anti-PGA polyclonal antiserum in trichome (**A** and **C**) and epidermal (**B**) cells of expanding cotton leaves. **A** Trichome cell with pectin labelling throughout the wall, although not noted in the thick cuticular area (*c*). **B** In an epidermal cell from the same section as the trichome (**A**), the cell walls of the epidermal cells (*e*) are virtually unlabelled. *c* Cuticle. **C** Although some trichomes displayed labelling throughout the wall, others are predominantly labelled in a more electron-opaque region (*1*), with more sparse labelling in the inner (*2*) layer. *c* Detached cuticle. Bar: A and B, 1.0 μm, C, 0.5 μm


A
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Discussion

Pectins in cotton fibers

Pectins have long been noted in cotton fibers with both biochemical and cytochemical stains (Anderson and Kerr 1938, Whistler et al. 1940, Meinert and Delmer 1977). One of these early studies correctly guessed that the cellulose in the young fiber "is effectively insulated by pectic substances" (Anderson and Kerr 1938) on the basis of the inability to stain young fibers with stains specific for cellulose before the removal of the pectins. Most of the data collected by others support the appearance of pectin in young stages of development and its gradual loss (or relatively lower percentage of total fiber weight) as the fiber develops. Meinert and Delmer (1977) analyzed the chemical composition of fibers and also found pectins (as uronic acids) to be plentiful in developing cotton fibers. These authors determined an approximate 25% composition of pectins (uronic acids) in the fiber, which is supported here by the apparent 1/4 to 1/3 of the width of the cotton fiber wall as the electron-opaque pectin sheath in the early stages of development and a smaller amount in the relatively mature 18 DPA fibers. It should be noted that the various pectin antibodies recognize specific epitopes of pectin and it is these epitopic differences that are noted in their distribution in the inner and outer layers of the cotton fiber. Other pectin molecules, such as rhamnogalacturonans, are not labelled by these antibodies or sera and thus might assume a different distribution in the fiber than the PGA epitopes recognized by these sera. Indeed, preliminary data on rhamnogalacturonan-II distribution indicate a strong reaction along the plasmalemma–wall interface in cotton (Vaughn unpubl.). Possibly, even the presence of a pectin epitope could be masked by the presence of the cellulose present in the inner layer. However, we feel this is unlikely, as simple de-esterification of the sections with sodium carbonate results in strong labelling of the inner layer with both JIM5 and the polyclonal anti-PGA backbone serum, even though both fail to label this area without the sodium carbonate treatment (unpubl.). An alternative possibility, that the cellulose and xyloglucan are present in the outer layer but are obscured by the abundant pectins in that layer cannot be eliminated by our data.

The very separate nature of the walls of the cotton fiber with regards to the distribution of pectin and cellulose-xyloglucan also supports the hypothesis of Carpita and Gibeaut (1993) on the relatively independent nature of the cellulose-xyloglucan and pectin (or at least that fraction that is highly de-esterified) elements within the wall. In the Carpita and Gibeaut (1993) model, the two networks are seen as separate but intermeshed whereas, in the cotton fiber, the layers occupied by either component occur mutually exclusive within the wall. In other tissues within the boll, the middle lamella is the principle site of de-esterified pectin accumulation and occurs peripheral to the cellulose-xyloglucan component as well. Localizations with the JIM7 antibody to highly esterified pectin epitopes reveals some labelling of both the inner and outer layers of the fiber cell wall, indicating that the esterified precursor form synthesized in the Golgi apparatus must cross the inner layer of the cell wall where it is de-esterified and assumes its final location. Also there may be bona fide esterified pectin components that interact with the cellulose-xyloglucan layer in the inner layer of the fiber, but they must be predominantly esterified ones.

Possible roles of the pectin sheath in fiber development

Why the fiber maintains this peripheral layer of pectin whereas other epidermal cells do not is an interesting question of development. In both cases, a cuticle layer extends over the wall, so both would be protected from dessication, a potential role for a pectin type gel. Moreover, pectins appear to have more and greater roles in cell differentiation than providing simple hydration. Pectins control the extensibility and expansion of cells in ripening fruit (reviewed in Goldberg et al. 1996) and it is likely that similar sorts of control would allow for fiber cell extensibility and elongation whereas neighboring, pectin-poor epidermal cells would lack such ability. A bilayered epidermal wall with asymmetric pectin distribution has also been described recently in epidermal cells of flax stems (Jauneau et al. 1997), pea stems (Fujino and Itoh 1998), and maize coleoptiles (Schindler et al. 1995). These studies reveal the presence of a pectin sheath is negatively correlated with the ability of these cells to elongate, whereas in cotton fibers and trichomes it is only those cells which contain a pectin sheath that do elongate. In cotton, the epidermal cells in bolls and leaves actually lack a pectin sheath, indicating a fundamental difference in these walls and those described by these other authors. Presently, we are investigating the effects of herbicides and mutations that might answer questions as to the role(s) of the various wall components in controlling elongation of the cotton fiber cells.

Acknowledgments

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